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TRANSDERMAL DELIVERY OF HYALURONIC ACID BY PULSED CURRENT IONTOPHORESIS

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Introduction

Hyaluronic acid (HA) is a natural polysaccharide with different biological activities. HA is used in treatment of skin diseases including radioepithelitis, venous leg ulcers, diabetic foot lesions and actinic keratoses (1). In addition, it is used as a filler agent for improving wrinkles, scars, increasing volumes (2) and rejuvenating the skin (3). In fact, HA products are the most commonly used fillers before collagens (4, 5). Nevertheless, its chemical structure and its molecular weight prevent it from permeating the epidermal barrier; for this reason, HA has to be injected by experienced operators. This way of administration limits the use of HA. Electrically-assisted methods have not yet been studied for their efficacy in delivering HA across the stratum corneum (SC). In this study we evaluated the efficacy of a powered iontophoresis drug delivery system approved for clinical use in humans by the U.S. Food and Drug Administration (FDA), in delivering HA through living rat skin.

Materials and Methods

Device for pulsed current iontophoresis.

The device used in this study (Mattioli Engineering, Firenze, Italy) is a powered iontophoresis drug delivery system (Figure 1) that is indicated for the transdermal administration of ionic solutions for medical purposes and can be used as an alternative to injections (FDA approval n. K042590, 10.14.2004).

Drug delivery protocol.

All the experiments (performed according to "Principles of Laboratory Animal Care") were performed using male Wistar rats, age 6-8 months, weight 350+/-50 g. Each experimental point was repeated three times (i.e. in three different animals). Rats were anaesthetized and depilated. The Manufacturer's instructions of the drug delivery system used in this study, recommend to perform a mild abrasion on the area to be treated to ensure repeatibility as a result of the standardization of the thickness of the SC. Abrasion was performed using a closed-loop system with corundum crystals. Skin impedance was measured after shaving, and after abrasion (i.e. before beginning iontophoresis). For the study of transdermal delivery of fluorescent HA, 5 mg of fluorescein isothiocyanate (FITC)-conjugated HA (Molecular Probes, Carlsbad, CA, USA), was dissolved in 2.5 ml of phosphate buffered saline (PBS) to have a final concentration of 2 mg/ml. 500 µl was applied on each of two selected skin areas (one at the left and one at the right side of spinal cord). Both areas (control and treated), underwent shaving and abrasion as indicated. For the study of transdermal delivery of radioactive HA, 2 mg of [^{3}H]-HA (250 mCi/g, 1 mCi/ml) (American Radiolabeled Chemicals, St. Louis, MO, USA) was resuspended in 1.0 ml of PBS to have a final concentration of 2 mg/ml; 500 µl was applied onto two seleced areas of the skin as described above.

Evaluation of transdermal delivery of fluorescent HA by confocal laser scanning microscopy and by light microscopy.

Full thickness biopies were obtained from treated and control areas. Sections were examined by a confocal laser scanning microscope (Bio-Rad, Hercules, CA, USA) equipped with a 15-mW Kr-Ar laser and with differential interference contrast (DIC) optics for transmission images. Fluorescence was collected using a Nikon PlanApo x20 and x40 lens objective. Series of optical sections at intervals of 0.4 µm were obtained and superimposed to create a single composite image.The laser potency, photomultiplier and pinhole size were kept constant. Specimens for light microscopy were stained by Haematoxylin-Eosin solution (Sigma Aldrich, Milan, Italy) and images, at different magnification, were captured by a digital camera connected to the microscope.

Measurement of transdermal delivery of radioactive HA.

Full thickness biopsies were taken both from treated and from control areas. Separation of dermis from epidermis was performed incubating the skin in a 25 caseinolytic unit/ml of Dispase (Sigma Aldrich, Milan, Italy) in Hank's Balanced Salt Solution with Gentamicin (5 µg/ml) (Sigma Aldrich, Milan, Italy) for 6 h at 37°C. Radioactivity (cpm) was measured by a liquid scintillation counter. Average biopsy weight was 250 mg. Presented data are means +/- S.E.M. (n=3). Statistical analysis was performed using Student's t test. Values were significantly different with $p < 0.05$.

Figure 1. Head of the electric device used to perform pulsed iontophoresis. Actual size: 30 mm dia.

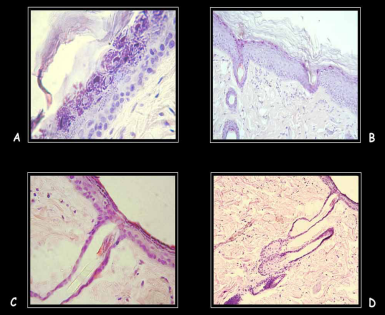

Figure 2. Control and treated rat skin. Untreated skin (control) showed a thick SC (panel A); epidermis, dermis and adnexa were evident (panel B). Treated skin showed a SC reduced in thickness (panel C); epidermis, dermis and adnexa appeared unaffected (panel D). Haematoxylin-Eosin staining. Total magnification x20 and x40.

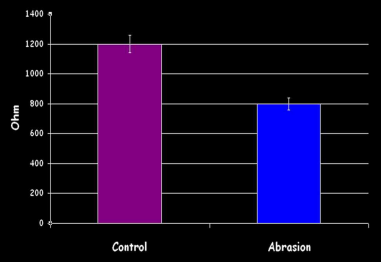

Figure 3. Measurement of rat skin impedance before and after mild abrasion.

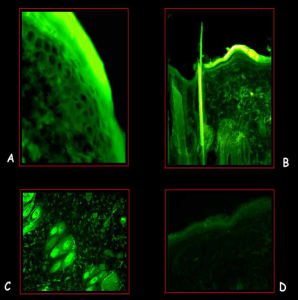

Figure 4. Fluorescent HA distribution in rat skin. After pulsed iontophoresis fuorescent HA was distributed on the SC and in the underlying epidermis layers (panel A). Fluorescent HA was also detected in the dermis where it appeared evenly distributed (panel B and C). Untreated skin showed only a weak fluorescence background (panel D). Confocal laser scanning microscopy. Total magnification x20 and x40.

Table 1

Transdermal delivery of radioactive HA

	Control	Treated (pulsed iontophoresis)
Applied	233,721 +/- 1,322	235,813 +/- 1,415
Recovered	825 +/- 69	22,918 +/- 772
Epidermis	-----	16,964 +/- 713
Dermis	-----	5,654 +/- 191

Table 1. After pulsed current iontophoresis about 10% of radioactive HA applied (cpm) onto the skin was recovered in biopsies. About 25% of [^{3}H]-HA associated to treated skin was distributed in the dermis and the rest in the epidermis. [^{3}H]-HA associated to control areas was not significant.

Results

Observation by light microscopy after Haematoxilyn-Eosin staining, showed no significant difference between treated and control skin areas (Figure 2). Control (Figure 2, panel A and B) refers to an area that had been depilated and shaved, but where no abrasion or electric treatment had been performed. On the surface of both specimens, the SC could be observed,and it appeared thick and well preserved. The thickness of the SC was decreased in treated areas because of abrasion (Figure 2, panel C and D). However, we could not detect any lesion to this layer or to the underlying layers of the epidermis. Epidermis, dermis and appendages, such as hair follicles and sebaceous glands, did not show any significant modification in shape, consistency, or staining. No morphological sign of tissue injury could be detected in the treated samples. Determination of skin impedance (Figure 3) provided results consistent with those reported above. As expected, abrasion significantly reduced rat skin impedance. However, the value of electric impedance measured after abrasion was still suggestive of skin barrier integrity. Pulsed current iontophoresis allowed the transdermal transport of fluorescent HA. The distribution of fluorescent HA immediately after pulsed current iontophoresis is shown in Figure 4 (panel A, B, and C). Fluorescent HA was detectable both in the SC and in the underlying epidermis layers. Fluorescence in the epidermis was distributed along inter-cellular pathways so to clearly evidence both the outline of the corneocytes forming the SC and the outline of the keratinocytes in the underlying layers (Figure 4, panel A). Fluorescence was also observed in the dermis, demonstrating a diffusion of fluorescent HA through the dermo-epidermal junction (Figure 4, panel B and C). Since hair follicles and their sheaths represent a physiological channel from outside to he deepest skin layers, fluorescence was also detected in association to these structures (Figure 4, panel B). In control areas (Figure 4, panel D) only a weak background fluorescence was detectable in the epidermis and dermis. Next, we measured the transdermal transport of radioactive HA. Table 1 shows hat spontaneous (control, i.e. without electric treatment) transdermal passage of radioactive HA was not significant. After pulsed current iontophoresis, we could observe a significant amount of radioactivity recovered in the biopsies, reflecting electrically-driven transdermal delivery of HA. In the biopsies from treated areas we recovered about 10% of the radioactive HA applied onto the skin. Analysis of radioactive HA distribution showed that about 25% of radioactive HA in the skin was associated to the dermis; the rest was recovered in the epidermis.

Discussion

HA is a polysaccharide with a variety of biological properties related to wound healing, blood coagulation and cancer biology. In plastic surgery and in cosmetic dermatology it is used as a soft tissue filler for several procedures. When applied as a gel onto the skin, HA localizes in the epidermis where potential receptors for HA were described. For this reason, the only way of administering HA as a soft filler is by injection.This hindrance contrasts with the needs of today's demanding world that ask for fast and painless "lunchtime procedures". Here we propose that pulsed current iontophoresis could be used as alternative to injection to deliver HA through the skin, in particular into the dermis. Furthermore, it should be considered that being the entire treatment fast and painless and avoiding all the practical difficulties associated with syringes and needles, it could be repeated several times without complications. We hypothesize that the association of the safest filler available with a non surgical, quite simple procedure such as iontophoresis, could satisfy the request for rapid and painless treatment.

References

1. Weindl et al., 2004. 2. Narins et al., 2005. 3. Andre, 2004. 4. Dover et al., 2005. 5. Ascher at al., 2004.

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